tion (anemic dermography) measures the adrenin content in the blood stream."

It will be noted that this conception is diametrically opposed to Sergent's belief, which would have the white line reflect a paucity of adrenalin in the blood stream.

Conclusions. 1. From the study of a series of 255 cases of a variety of diseases and normals, upon which numerous pharmacologic and other tests were performed, we feel justified in asserting that the so-called white adrenalin line of Sergent is a local vasomotor reflex, resident in the skin, bearing no direct relationship to adrenal gland activity.

2. The reasons for postulating the above are: (*a*) Its independence of blood-pressure, acute fatigue and other signs of hypo-adrenia; (*b*) its frequent occurrence in normals and in a variety of diseases unassociated with hypo-adrenia; (*c*) its reappearance in the face of persistent general manifestations of adrenalin subcutaneously administered; (*d*) its peculiar association with scarlet fever.

3. It would appear that the state of the vasomotor system which allows of its best exhibition is found in young adults of either sex, and especially in the exanthem of scarlet fever.

4. On the basis of the above series it may be stated that this line has not the clinical significance attributed to it.

5. In spite of the various hypotheses evolved regarding it, further work seems necessary to establish the exact physiologic mechanism of this remarkable vasomotor phenomenon.

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CHOLESTEROL IN CEREBROSPINAL FLUID.

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Recently some importance has been attached to the presence of cholesterol in cerebrospinal fluid by Pighini[1] and by Hauptmann.[2]

[1] Ueber den Cholesteringehalt der Lumbalflüssigkeit einiger Geisteskrankheiten, Hoppe-Seyler's Ztschr. f. physiol. Chem., 1909, lxi, 508.

[2] Eine biologische Reaktion im Liquor Cerebrospinalis bei organischen Nervenkrankheiten, Med. Klin., 1910, vi, 181.

In view of this claim and in view of the role of blood cholesterol in various diseases we have made a series of determinations of cholesterol in cerebrospinal fluid, with special attention to pathologic fluids.

Methods.—The method of Bloor,[3] with a few modifications, has been employed by us for quantitative determination and the method of Hauptmann[2] for qualitative determination. It should be noted here that although Hauptmann himself was not certain that the inhibition of saponin hemolysis by cerebrospinal fluid in some pathologic cases was due to the presence of cholesterol a comparison of the saponin with the Bloor method shows that with a few exceptions the two tests run quite parallel.

In the Bloor method the amount of fluid to be used for each test was naturally a question of great importance, as it was clear that cerebrospinal fluid could not contain as much cholesterol as blood contains.

After a series of experiments we found that whenever the cerebrospinal fluid contains cholesterol in appreciable amount, 3 c.c., the amount used for blood cholesterol will give a reading in the colorimeter. If 3 c.c. of fluid do not give a reading, 6 c.c. or even 10 or 15 c.c. of the same fluid will give no reading in the colorimeter. We therefore decided to take 3 c.c. as the standard amount of fluid, as one is usually able to spare this amount with ease. Whenever possible, however, 6 c.c. or more of the same fluid or of the same type of fluid were used as a check.

Three c.c. of the fluid which had been drawn by means of a spinal or ventricular puncture were measured into a 100 c.c. volumetric flask and ether-alcohol (1 to 3) added. The flask was heated to the boiling-point and set aside. After standing twenty-four hours to several days, ether-alcohol was added to the 100 mark, the contents filtered and 80 c.c. of the filtrate taken for the cholesterol determination. We found 10 c.c. of the filtrate, the amount used in the blood cholesterol method, insufficient for the determination of cerebrospinal cholesterol.

The filtrate was evaporated to dryness and the cholesterol extracted by chloroform and the chloroform extract concentrated to 5 c.c., which was then put into a 10 c.c. graduate, 2 c.c. acetic anhydride and 0.2 c.c. sulphuric acid added, shaken and put away for fifteen minutes in a dark place. The material was then compared with a standard of 0.5 mgm. of cholesterol in a Duboscq colorimeter if the color of the cerebrospinal filtrate were sufficient to make a reading. If no color at all appeared the solution remained light yellow and we called it negative. If a greenish-yellow color appeared we designated it as a trace.

[3] The Determination of Cholesterol in Blood, Jour. Biol. Chem., 1916, xxiv, 227. The Determination of Cholesterol in Blood, Jour. Biol. Chem., 1917, xxix, 437.

The technic of the Hauptmann test used was as follows: Each time the test was performed the hemolytic unit of saponin was determined. Various samples of saponin tried were found to vary greatly in their hemolytic action, but the sample finally used caused hemolysis in a 1 to 10,000 dilution. The dosage of this dilution necessary to cause complete hemolysis of 0.5 c.c. of a 7 per cent. suspension of washed sheep erythrocytes in 0.85 per cent. saline was determined by adding increasing amounts of the saponin solution to the corpuscle suspension in a series of small tubes. The hemolytic unit of the 1 to 10,000 saponin solution was usually found to be 0.3 c.c.; occasionally it was 0.2 or 0.4 c.c. After the saponin unit has been determined, 0.8 c.c. of cerebrospinal fluid to be tested was set up with the required amount of saponin and 0.5 c.c. of 7 per cent. sheep red corpuscle suspension. Control tubes with normal cerebrospinal fluid (one known not to produce any inhibitory effect on saponin) and with normal salt solution were also set up. The results were read after ten minutes in the water-bath at 37° and again after twelve hours in the refrigerator. The setting up of the saponin test with a positive result is illustrated by the following table:

Tube 1. 1 to 10,000 saponin, 0.3 c.c.; normal cerebrospinal fluid, 0.8 c.c.; 7 per cent. sheep corpuscle suspension, 0.5 c.c. Result: Complete hemolysis.

Tube 2. 1 to 10,000 saponin, 0.3 c.c.; cerebrospinal fluid to be tested, 0.8 c.c.; 7 per cent. sheep corpuscle, 0.5 c.c. Result: Inhibition of hemolysis.

Tube 3. 1 to 10,000 saponin, 0.3 c.c.; 0.85 per cent. saline, 0.8 c.c.; 7 per cent. sheep corpuscle suspension, 0.5 c.c. Result: Complete hemolysis.

Results.—We have examined 168 fluids for cholesterol; 74 of these were examined quantitatively, and of these 52 were also examined by the saponin method. The rest of the fluids were examined only qualitatively by the saponin method. Our fluids divided themselves into six groups:

1. *Normal fluid*, including all fluids in which the Wassermann, Lange, cell count and globulin tests were negative. These include a variety of conditions, such as pneumonia, multiple sclerosis, amaurotic family idiocy, epilepsy and other cases in which irritation of the brain justified spinal puncture but the fluid showed no pathologic findings; 88 fluids of this type were examined. Of these 24 were examined both quantitatively and qualitatively and 64 only qualitatively.

2. *Luetic or Neurosyphilitic Fluid.* This includes all fluids in which the Wassermann and Lange reactions were found positive. Of these 25 fluids were examined both quantitatively and qualitatively and 28 fluids only qualitatively.

3. *Meningitic fluid*, including all forms of meningitis. There were 16 fluids in this group examined both quantitatively and qualitatively.

4. *Brain tumor* (4 cases) and *brain abscess* (2 cases). Of the brain tumor cases a positive diagnosis had been made in 3 and a probable diagnosis in 1. The fluid from 2 of the positive brain tumor cases and from the one suspicious case were examined both quantitatively and qualitatively; that from 1 positive case only qualitatively. The fluid from 1 case of brain abscess was examined both quantitatively and qualitatively and the other only qualitatively.

5. *Hemorrhage of the Brain*. Two cases, both examined by both methods.

6. *Ventricular Fluid*. Three cases, both methods being used in each case.

Normal fluid gave no reaction at all with the Bloor method, no matter whether 3, 6 or even 10 c.c. were used; only when 25 c.c. of fluid was taken for a test was there a trace of cholesterol present. Saponin hemolysis was not inhibited by the presence of normal cerebrospinal fluid.

Luetic fluid, with three exceptions, also gave no reading with the Bloor method nor was there any inhibition of hemolysis. Of the three fluids that contained cholesterol in appreciable amounts one was also meningitic in character. The two others were diagnosed merely as general paresis.

Of the fluid from cases of meningitis only three contained a sufficient amount of cholesterol to enable us to read it in the colorimeter, one of these also giving a positive Wassermann reaction. The rest of the fluids showed a greenish-yellow discoloration as the end reaction, which was taken to indicate the presence of a trace of cholesterol. There was not, however, a sufficient amount of cholesterol in them to allow a reading in the colorimeter. Saponin hemolyzed blood in the presence of meningitic fluid, although occasionally the hemolysis was delayed.

Brain tumor allowed no reading with the Bloor method, although a trace was present. In 2 cases saponin gave a partial or delayed hemolysis, also indicating a trace of cholesterol. In the other case no inhibition was present with saponin. One case of brain abscess gave a reading of 13 mgm. of cholesterol per 100 c.c. of fluid, but no inhibition of hemolysis took place. It may be interesting that the above case of brain abscess also had a meningitis which seemingly was terminal, as no meningeal symptoms were present throughout the disease. The cholesterol could not be due to the meningitis, however, as most cases of meningitis showed only a trace of cholesterol which could not be read in the colorimeter. Saponin caused hemolysis in the presence of the fluid from this case. The fluid from the other case of brain abscess was examined only for saponin hemolysis; hemolysis was delayed but not completely inhibited.

The 2 cases of hemorrhage of brain under observation both gave a large amount of cholesterol, the amount being 8 mgm. per 100 c.c. of fluid in one case and 17 mgm. in the other. In both cases there was complete inhibition of saponin hemolysis. The fluid in the latter case was removed from the ventricles of the brain and was yellowish in color. In the other case the fluid was removed by spinal puncture and was colorless.

Of the three ventricular fluids examined one was a hemorrhage of the brain, and, as mentioned above, gave a high cholesterol reading in the colorimeter (17 mgm.) and a complete inhibition of saponin hemolysis. The other ventricular fluid, which also gave a high cholesterol reading (103 mgm.) and complete inhibition of saponin hemolysis, was obtained from a premature infant with a marked hydrocephalus. The appearance of the ventricular fluid, the high cell count with the predominance of polymorphonuclear leukocytosis and a strong globulin reaction make the diagnosis of a meningitis certain, although the type of the meningitis could not be diagnosed, as no organism was isolated from the fluid. The great amount of cholesterol in the fluid makes one suspect the presence of a hemorrhage of the brain in the case.

The third ventricular fluid was obtained from a case of hydrocephalus and gave no reading in the colorimeter and no inhibition of saponin hemolysis. This last fluid shows that under normal conditions, *e. g.*, when there is no hemorrhage or meningitis, ventricular fluid contains no cholesterol.

Discussion.—In the older literature there is only an occasional reference to cholesterol in cerebrospinal fluid. Thus Schlossberger,[4] Zdarek,[5] Panzer[6] and Coriat[7] speak of the presence of cholesterol in hydrocephalus fluid. Of late four attempts have been made to study cholesterol in various types of cerebrospinal fluid systematically. Pighini[8] found cholesterol in a large percentage of fluids of general paresis (88 per cent.), of epilepsy (66 per cent.) and of dementia precox (43 per cent.). Hauptmann[9] found that saponin hemolysis is inhibited by cerebrospinal fluid from cases of brain and cord tumor (100 per cent.); hemorrhage of the brain (85.7 per cent.), tabes (83 per cent.), cerebrospinal lues (65 per cent.), multiple sclerosis (46 per cent.). He thought that the inhibition is due to the presence of cholesterol. Chaufford, Laroche and Grigant[10] and Weston[11] found small amounts of cholesterol even in normal fluid.

[4] Analyse von hydrocephalischen Flüssigkeiten, Arch. f. physiol. Heilk., 1851, x, 581.

[5] Ein Beitrag zur Kenntniss der Cerebrospinalflüssigkeit, Ztschr. f. physiol. Chemie, 1902, xxxv, 202.

[6] Zur Kenntniss der Cerebrospinalflüssigkeit, Wien. klin. Wchnschr., 1899, xii, 805.

[7] The Chemical Findings in the Cerebrospinal Fluid and Central Nervous System in Various Mental Diseases, Amer. Jour. Insanity, 1903–4, lx, 733.

[8] Loc. cit.

[9] Loc. cit.

[10] Le taux de la cholesterine dans de liquide cephalorachidien normal et pathologique, C. R. de Soc. de Biol., 1911, lxx, 146.

[11] Cholesterol Content of Cerebrospinal Fluid, Jour. Med. Research, 1915, xxxiii, 119.

Pighini determined cholesterol qualitatively by the Liebermann method, Hauptmann studied it by a serological method, while Chaufford, Laroche and Grigant and Weston determined it quantitatively.

The methods used by the above workers varied. Pighini used 25 c.c. of fluid and employed a tedious and complicated method. Chaufford, Laroche and Grigant unfortunately do not give the method they employed nor how much fluid they used. Weston worked with postmortem fluid in very large quantities, this method being open to criticism, on the ground that cerebrospinal fluid is known to undergo chemical and physicochemical changes post mortem.

Our results do not agree with those of Chaufford, Laroche and Grigant or with those of Weston, in that we found no cholesterol at all in normal fluids. Nor do our results coincide with those of Pighini, who found cholesterol in a very large percentage of fluids with a positive Wassermann reaction.

Our cases of brain tumor have been too few in number to express an opinion as to the accuracy of Hauptmann's statement. With the Bloor method there was only a trace of cholesterol in the two cases of brain tumor in which this method was used, and with the saponin method there was partial inhibition. The one case of brain abscess examined quantitatively gave a high reading of cholesterol in the colorimeter. There was, however, no distinct inhibition of saponin hemolysis, nor was there marked inhibition in the fluid from brain abscess examined only qualitatively.

Our results in hemorrhage of the brain agree with those of Hauptmann, there being a large amount of cholesterol with the Bloor method and complete inhibition of saponin hemolysis.

With minor exceptions the saponin method of Hauptmann agreed fairly well with the Bloor method, although the latter is more quantitative and more sensitive. This also shows that Hauptmann was right in his contention that the inhibition of hemolysis in some fluids is due to cholesterol.

The above results, we believe, show that the amount of cholesterol in pathologic cerebrospinal fluid depends wholly or in part on the degree of permeability of the meninges and has no specific pathogenesis. This is evident by the presence of a trace of cholesterol in all cases of meningitis and by the presence of large amounts in hemorrhage of the brain. It is also evident from our work that the claim of Pighini of the dependence of a positive Wassermann reaction on cholesterol has no foundation.

The usefulness of the cholesterol determination in cerebrospinal fluid for diagnostic purposes, we believe, is limited because of its presence in hemorrhage of the brain, in some cases of meningitis and also occasionally in general paresis. Still, its presence is corroborative evidence of the existence of a pathologic process producing increased permeability of the meninges. Whenever the

history of the case indicates it, hemorrhage of the brain should therefore be thought of, especially when large amounts of cholesterol are present.

Conclusions.—Normal cerebrospinal fluid contains no cholesterol or only a very small trace of cholesterol.

Fluid in which the Wassermann and Lange reactions are positive contains no cholesterol in appreciable amounts. Only three out of twenty-five such fluids gave a reading in the colorimeter.

Fluid of hemorrhage of the brain showed high cholesterol content.

Fluid from tumor of the brain gave a trace of cholesterol.

Fluid from a case of brain abscess gave a high cholesterol reading.

The majority of meningitis fluids showed a trace of cholesterol. Three fluids had a high reading.

Ventricular fluid gave no cholesterol reading, except when there was the presence of hemorrhage of the brain or other pathologic condition.

The Hauptmann reaction seems to depend on the cholesterol content of the cerebrospinal fluid.

This work does not bear out Pighini's contention that the Wassermann reaction depends on the cholesterol of the fluid.

We believe that the cholesterol content depends wholly or partially on the permeability of the meninges.

CASES IN WHICH CHOLESTEROL WAS FOUND IN APPRECIABLE AMOUNTS BY THE BLOOR METHOD.

Name.	Diagnosis.	Quantity of fluid used.	Wassermann.	Cholesterol (Bloor) mgm. per 100 c.c.	Saponin.	Remarks.
P. P.	General paresis	6.0 c.c.	++++	13.3	Complete hemolysis	
Mixture	General paresis	10.0 c.c.	++++	6.2	Delayed hemolysis	
E. C.	Pneumococcus meningitis	3.0 c.c.	Negative	19.0	Inhibition in water-bath; hemolysis in twelve hours	Fluid drawn while patient was moribund.
M. N.	Tuberculous meningitis	5.0 c.c.	Negative	10.2		
S. S.	Meningitis	2.3 c.c.	++++	14.1	.....	Type of meningitis undetermined.
L. P.	Cerebral hemorrhage	3.0 c.c.	Negative	8.0	Partial inhibition	
B. A.	Meningeal hemorrhage; meningitis	3.0 c.c.	Negative	17.0	Inhibition	Ventricular fluid.
B. S.	Hydrocephalus; meningitis; meningeal hemorrhage ?	3.0 c.c.	Negative	103.0	Complete inhibition	Ventricular fluid.
J. C.	Brain abscess; terminal meningitis	6.0 c.c.	Negative	13.0	Complete hemolysis	Native amboceptor present.